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Modifier genes play a significant role in the phenotypic expression of PKD1[1]

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Modifier genes play a significant role in the phenotypic expression of PKD1.

Background. Polycystic kidney disease type 1 (PKD1) is characterized by extreme variation in the severity and progression of renal and extrarenal phenotypes. There are significant familial phenotype differences; but it is not clear if this is due to differences in *PKD1* mutations, differences in genetic background, or both.

Methods. A total of 315 affected relatives (83 PKD1 families) without end-stage renal disease (ESRD) were evaluated for disease markers, including renal volume, creatinine clearance, proteinuria, liver cysts, and hypertension. Of these patients, 19% progressed to ESRD within 1 to 10 years after the initial examination. Nested analysis of variance was used to investigate interfamilial and intrafamilial differences in these phenotypes. Heritability analyses were used to estimate the effect of the genetic background on phenotypic variability. The age of onset of ESRD was also analyzed with an additional 389 family members from the same PKD1 families without clinical evaluation but with data on age of onset of ESRD (or age without ESRD).

Results. There were significant phenotype differences between patients with the same mutation and different genetic backgrounds. The phenotypic variation between patients with different mutations and different genetic backgrounds was not significantly greater than the variation between patients with the same mutation and different genetic backgrounds. However, when the 389 family members were included, both the mutation and modifier genes had significant effects on the age of onset of ESRD. Inherited differences in genetic background were estimated to account for 18% to 59% of the phenotypic variability in PKD1 disease markers in patients prior to ESRD and in the subsequent progression to ESRD (43% heritability) in the 315 patients who were clinically evaluated.

Conclusion. Modifier loci in the genetic background are important factors in inter- and intrafamilial variability in the phenotypic expression of PKD1. The extreme intrafamilial phenotype differences are consistent with the hypothesis that one or a few modifier genes have a major effect on the progression and severity of PKD1.

Autosomal-dominant polycystic kidney disease (ADPKD) is one of the most common inherited conditions in humans [1]. The disease is caused by a mutation in either the *PKD1* or the *PKD2* gene, with *PKD1* mutations accounting for over 80% of patients [2]. Multiple renal epithelial cysts and progressive renal enlargement are typically present in mutation carriers over 30 years of age [3]. Beyond these hallmarks of the disease, there is tremendous variability in the phenotypic expression of the primary genetic defect in ADPKD. Liver cysts are the most common extrarenal manifestation, occurring in at least 75% of patients by age 60 years [4]. Hypertension occurs in 50% to 75% of patients prior to renal insufficiency, and is thought to accelerate the decline in renal function [5–10]. Although recent studies have shown significant slowing of ADPKD renal progression over the last 2 decades, approximately 50% of ADPKD patients progress to end-stage renal disease (ESRD) by age 60 years [7, 10]. The extreme variability in renal function, presentation of liver cysts and hypertension, and rate of progression to ESRD has made it difficult to predict prognosis in individual patients. The factors contributing to the variability remain largely unknown.

A number of studies have documented significant interfamilial and intrafamilial phenotype variation in ADPKD [11–14]. Differences between families are partly due to locus heterogeneity, with progression to ESRD delayed by 10 to 20 years in patients with *PKD2* mutations compared to patients with *PKD1* mutations [14–17]. However, there are also significant differences in the age at onset of ESRD amongst PKD1 families. This observation cannot be explained by locus heterogeneity, since all of these families harbor mutations in the *PKD1* gene. The observed differences could reflect the diverse spectrum of defects in *PKD1* [13] and/or inherited variation in the

[1]**See Editorial by Pei, p. 1630.**

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structure and regulation of genes that modify the effects of the primary genetic defect [14].

Early studies of *PKD1* mutations failed to reveal any correlation between the characteristics of the molecular defect and phenotypes in ADPKD; however, several recent studies suggest an association between disease severity and the position of the mutation in *PKD1* [18–20]. With regard to modifier genes, studies in animal models of PKD have consistently shown that the nature and severity of renal abnormalities associated with a specific mutational defect are strongly dependent on the genetic background in which the mutation is found [21–26]. Limited data for ADPKD in humans, such as reports of racial differences in the severity [27], and extreme variation in severity among relatives with the same mutational defect [28–30], also support the conclusion that genetic modifiers are an important source of phenotypic variability in ADPKD. In the present study, we investigate the contributions of mutation diversity and allelic variation in modifier genes to phenotypic variability using a novel analysis for separating these effects.

METHODS

Study population

From June 1985 to December 2003, 848 ADPKD subjects from 397 families were recruited for a longitudinal study of the natural history of ADPKD at the University of Colorado Health Sciences Center (UCHSC). Patients who were in ESRD at the time of family contact were excluded from natural history studies. Blood or mouthwash DNA samples were obtained for genetic testing for *PKD1* and *PKD2* markers in affected and at-risk relatives and their spouses. The criterion for diagnosing ADPKD was taken as a single renal cyst by ultrasonography in children under the age of 18 years and bilateral renal cysts by ultrasonography in adults [3, 31]. All subjects provided written informed consent in accordance with protocols approved by the Colorado Multiple Institutional Review Board.

Genetic linkage analysis for family classification as PKD1 or PKD2 was performed in linkage-informative families using up to 18 polymorphic markers for *PKD1* and up to 12 polymorphic markers for *PKD2*. Multipoint parametric linkage analysis was performed with Allegro [32] using age-dependent penetrance estimates obtained by age of onset analyses using the Ageon routine of SAGE [33]. The posterior probability of *PKD1* linkage was calculated as:

$$P = 0.84 * 10^{L(PKD1)} / [0.84 * 10^{L(PKD1)} + 0.14 * 10^{L(PKD2)} + 0.02 * 10]$$

where L(PKD1) is the logarithm of the odds (LOD) favoring *PKD1* linkage and L(PKD2) is the LOD favoring *PKD2* linkage. The formula assumes prior probabilities of 0.84 and 0.14, respectively, for *PKD1* and *PKD2* linkage, and allows for the possibility of a third ADPKD locus in 0.02 of families. The criterion for classifying a family as PKD1 by linkage analysis was taken as $P > 0.97$. Seventy families met this criterion. Fifteen additional families were classified as PKD1 based on previous [18] and ongoing (unpublished) screening for mutations in the PKD1 gene (Table 1).

A total of 315 PKD1-affected relatives from 83 families participated in natural history studies. None of these patients required renal replacement therapy at the time of enrollment and testing as part of the natural history study, which involved a 2-day visit at the UCHSC General Clinical Research Center (GCRC). Complete physical examinations were performed on each participant, blood pressure was measured by a trained nurse, blood samples were drawn for serum chemistries, 24-hour urine samples were collected, family and medical histories were obtained, and renal and liver ultrasound examinations were performed in the UCHSC Radiology Center. Follow-up studies to determine subsequent development of hypertension or progression to ESRD were conducted by telephone interviews and mail-in questionnaires. Another 389 relatives from these same families were not enrolled in the natural history studies and therefore did not have data from a GCRC visit. Of these 389 patients, 180 (46%) had reached ESRD. The age of onset of ESRD was analyzed both with and without these relatives.

Phenotype definitions

Serum creatinine, creatinine clearance, urinary protein excretion, renal volume, and the presence/absence and number of liver cysts were assessed during the 2-day clinic visit. The presence/absence and age at diagnosis of hypertension was determined at the time of the clinic visit, or upon follow-up for patients who were initially normotensive. All of the information on subsequent progression to ESRD for these patients was obtained after the initial clinic visit.

Renal volume was defined as the mean of both kidneys and was calculated using a standard formula for a modified ellipsoid for each kidney as follows:

$$\text{renal volume} = \frac{4}{3}\pi \times \left(\frac{\text{anteroposterior diameter}}{4} + \frac{\text{width}}{4}\right)^2 \times \frac{\text{length}}{2}.$$

The presence of liver cysts at a given age was determined by ultrasound. Liver cyst phenotypes were defined as the presence/absence or number (0, 1 to 5, 6 to 15, >15 cysts) of liver cysts at the time of examination. Urinary protein concentrations were determined by the Coumassie blue dye-binding method. The mean of two collections was taken as the level of proteinuria (mg/24 hours). A serum creatinine measurement and two consecutive 24-hour urine collections were obtained for determination of creatinine clearance normalized to

Table 1. Summary of family sizes and structures, and documentation for classifying 83 *PKD1* families

Family number	Family size: Number of sibships ≥1 affected	Number of sibships ≥1 examined	Number of affected Total	Number of affected Examined	Number with ESRD Total	Number with ESRD Examined[a]	PKD1 documentation Linkage[b] or mutation [L (*PKD1*); L (*PKD2*)]
24	3	2	8	7	1	0	0.99 (1.93; −2.99)
41	3	2	6	3	0	0	0.99 (0.93; −4.14)
43	6	4	10	8	1	0	0.99 (3.44; −5.72)
45	6	4	11	6	2	1	0.99 (2.16; −1.05)
46	4	3	6	3	1	0	R2392P[c]
47	4	3	9	4	4	1	0.99 (3.63; −6.93)
62	6	4	9	5	4	1	0.99 (1.79; −1.83)
68	5	3	9	5	3	2	0.99 (1.95; ND)
72	3	3	6	5	2	1	0.99 (1.2; −4.76)
73	3	2	4	3	1	1	0.99 (1.32; −1.83)
74	8	4	11	5	3	1	0.99 (1.13; ND)
76	8	5	15	6	5	1	0.99 (2.6; ND)
82	4	2	6	4	2	1	0.99 (2.17; −2.16)
84	1	1	2	1	0	0	1671stop[d]
91	4	2	9	4	3	1	0.99 (1.27; −0.74)
98	6	4	10	5	4	2	0.99 (1.44; −1.25)
100	3	2	5	3	3	1	0.99 (0.81; −0.46)
109	15	12	32	17	12	3	0.99 (2.79; −5.26)
110	10	5	16	5	4	0	0.99 (1.32; ND)
119	3	2	4	2	3	1	0.98 (0.89; ND)
120	2	1	2	1	2	1	1306stop[d]
121	3	2	4	2	2	0	0.99 (1.04; −1.06)
124	3	2	6	4	1	0	0.99 (0.98; ND)
125	7	3	10	4	5	2	0.99 (3.16; −5.20)
138	4	3	6	3	2	2	1671stop[c]
165	5	3	16	6	9	2	0.99 (2.73; −1.34)
174	3	1	5	2	0	0	0.99 (1.14; −0.21)
177	5	3	8	3	0	0	0.99 (0.6; −1.64)
186	3	2	3	2	2	1	Y1517X[d]
192	2	1	7	5	2	1	0.99 (1.67; −1.35)
205	5	3	8	4	3	1	0.99 (0.71; −0.66)
256	4	2	6	3	3	0	0.99 (1.58; −2.68)
290	3	1	4	1	1	0	0.99 (1.48; −1.41)
294	5	3	8	4	4	2	0.99 (3.43; −3.44)
344	4	3	8	3	7	2	0.99 (0.86; −0.77)
349	5	2	9	3	3	1	0.99 (0.90; −2.13)
362	7	4	9	5	4	1	0.99 (1.39; ND)
371	4	2	8	4	3	3	0.98 (1.04; ND)
375	3	1	5	2	4	2	0.98 (0.53; −0.49)
386	5	2	6	2	3	0	Q3738X[d]
435	7	1	8	1	6	1	0.98 (1.06; 0.23)
459	3	3	5	5	0	0	0.99 (0.71; −0.66)
474	4	3	7	3	3	1	0.99 (1.27; ND)
519	4	3	7	5	0	0	S2423F[c]
523	3	2	6	4	1	1	0.98 (0.54; −0.56)
524	5	3	10	5	1	0	0.99 (2.35; −4.06)
525	3	2	7	6	0	0	0.99 (1.92; −1.99)
531	3	2	4	3	2	1	0.99 (1.36; −0.99)
558	5	3	9	7	1	1	0.99 (2.33; −1.60)
576	4	3	4	3	2	2	0.99 (0.83; −1.37)
579	13	5	31	6	8	1	0.99 (2.70; −2.66)
581	9	1	17	1	8	1	Q3701X[d]
584	3	2	6	5	1	0	0.99 (1.12; −1.27)
586	2	2	3	3	0	0	0.99 (0.30; −1.72)
588	4	3	13	5	5	1	0.99 (1.39; ND)
591	8	6	9	7	2	1	0.99 (2.12; −2.29)
834	4	3	6	4	2	1	0.99 (1.97; −4.13)
1015	3	2	4	3	3	2	0.99 (0.30; −2.20)
1167	5	3	9	4	2	0	0.99 (1.50; −0.62)
1202	2	2	2	2	0	0	L3510V[d]
1203	1	1	1	1	0	0	1365stop[d]
1205	4	1	10	4	6	2	0.99 (0.60; −0.81)
1245	4	2	7	3	0	0	0.99 (0.25; −2.23)
1283	8	3	12	3	4	1	0.99 (0.38; −3.13)
1296	3	1	4	1	3	1	1671stop[d]
1308	4	3	8	6	2	1	0.99 (1.51; 0.16)

Table 1. (Continued)

1313	9	2	20	3	9	0	0.99 (1.72; −0.89)
1334	4	2	7	2	5	1	1671stop[c]
1347	4	2	4	2	1	0	0.99 (1.19; −0.76)
1348	4	2	7	4	1	0	0.99 (0.82; −0.54)
1350	6	2	10	3	6	1	1671stop[d]
1359	16	2	26	3	7	0	0.99 (1.37; −3.72)
1732	4	2	6	3	1	0	0.99 (1.15; −3.23)
1757	4	1	6	2	3	0	0.99 (0.63; −2.03)
1816	6	2	13	3	4	0	0.99 (0.35; −2.72)
1884	4	2	9	3	2	0	0.97 (0.74; ND)
1915	5	1	9	2	4	0	0.99 (0.59; −2.08)
2356	2	1	3	2	1	0	0.99 (0.11; −2.21)
2421	5	2	12	3	4	1	0.99 (0.30; −2.21)
2440	4	2	9	6	3	0	0.99 (0.80; −2.73)
2874	2	2	5	5	0	0	0.99 (0.90; −1.53)
2888	2	1	8	2	5	0	0.99 (0.29; −2.14)
2967	5	3	7	3	2	0	0.99 (0.30; −2.14)

ESRD is end-stage renal disease.
[a]Reported to have developed ESRD 1 to 10 years subsequent to the clinic visit for the natural history study.
[b]ND is not done; information for all affected and unaffected relatives was included in linkage analyses.
[c]Watnick et al, 1999 [18].
[d]This laboratory.

1.73 m^2 body surface area. Hypertension was defined as blood pressure ≥140/90 mm Hg, a history of hypertension, or taking antihypertensive medication. ESRD was defined as the time of starting chronic dialysis treatment or receiving a renal transplant.

Age of onset phenotypes were defined in two ways. The first definition applied only to PKD1-affected relatives who had developed the condition or "event" (hypertension or ESRD). Age at onset was taken as the age at which hypertension or ESRD first occurred. Using this definition, the age at onset phenotype for event-free PKD1-affected relatives is considered unknown. The exclusion of the event-free relatives results in a truncated sample, which, in addition to reducing statistical power, may bias results. Therefore, a second phenotype was defined by fitting the marginal distribution of age at onset via a semiparametric model [34] implemented in the SAS procedure PHREG [35]. The PHREG procedure was used to fit a Cox proportional hazard model to age of diagnosis of hypertension and age of onset of ESRD, with gender included as a covariate. Based on a Wald chi-square test, these data were consistent with the assumption of proportional hazards. Martingale residuals from Cox regression were used as quantitative traits as described by others [36–38]. For the Cox model, the martingale residual for the ith subject with observation time t_i and event status δ_i, where $\delta_i = 0$ if t_i is a censored time or 1 if t_i is an event time, is:

$$\widehat{M}_i = \delta_i - \widehat{\Lambda}_0(t_i) \exp(\widehat{\beta}' z_i)$$

where $\widehat{\Lambda}_0(t)$ is the cumulative baseline hazard function evaluated at the age of onset of the event or, for event-free relatives, at the age at last examination [35].

The martingale residual for each subject was used as a score representing the liability of developing the phenotype by time (t). For example, an individual who developed hypertension at 19 years of age, or 14 years younger than the median age at diagnosis of hypertension (age 33 years), would have a positive score (0.937), whereas an individual who was still normotensive at age 55 years, or 22 years older than the median age at diagnosis of hypertension, would have a negative score (−1.66).

Statistical analyses

Analysis of variance (ANOVA) and heritability analyses were performed on eight different phenotypes: serum creatinine, creatinine clearance, urinary protein excretion, renal volume, presence/absence of liver cysts, number of liver cysts, age at diagnosis of hypertension, and age of onset of ESRD. The analyses were performed on each variable using gender, and, when appropriate, age at examination as covariates. Family and sibship within family were treated as random effects. Normalizing power transformations were performed to reduce skewness and/or kurtosis in the distributions of the variables, using the NOCOM computer program [39] to calculate the maximum likelihood estimate of the exponent, p. Statistical analyses were performed using the transformed variables, x^p, for each variable, x.

Two different ANOVA models were considered. The model 1 ANOVA takes the form:

$$Y = \mu + f_i + r_{j(i)};$$

where f_i represents the effects of family i, and $r_{j(i)}$ represents the effects of relative j within family i. The model 2 ANOVA takes the form:

$$Y = \mu + f_i + s_{j(i)} + p_{k(ij)};$$

where $s_{j(i)}$ represents the effects of sibship j within family i and $p_{k(ij)}$ represents the effects of person k within sibship j

Table 2. Disease markers and outcomes in natural history studies of 315 patients without end-stage renal disease (ESRD), and characteristics of the study population

	Males	Females	*P* value
Number	127	188	
Natural history studies[a]			
Age at visit *years*	33 ± 16	35 ± 16	NS
Renal volume cm^3	783 ± 621	596 ± 458	<0.005
Serum creatinine concentration *mg/dL*	1.8 ± 1.4	1.3 ± 1.0	<0.005
Creatinine clearance $mL/min/1.73\ m^2$	86 ± 42	82 ± 37	NS
Urinary protein excretion *mg/24 hours*	288 ± 448	228 ± 349	NS
No liver cysts %	55	50	
≥1 Liver cysts %	45	50	NS
1 to 5 Liver cysts %	15	17	
6 to 15 Liver cysts %	13	11	
>15 Liver cysts %	17	23	
Age at last follow-up years[b]	37 ± 17	38 ± 17	
Progressing to ESRD %	22	18	NS
Age at onset of ESRD *years*	50 ± 9 ($N = 28$)	54 ± 11 ($N = 33$)	NS
Presenting with hypertension[c] %	67	53	<0.05
Age at diagnosis of hypertension *years*	31 ± 11 ($N = 85$)	35 ± 13 ($N = 100$)	<0.05

[a]No patient was in ESRD at the time of the clinic visit.
[b]For subjects who progressed to ESRD, this is the age of onset of ESRD. For subjects who did not progress ESRD, this is the age when we were last informed that the patient had not reached ESRD (or age at death in the absence of ESRD).
[c]Hypertension may have been diagnosed before, during, or after the clinic visit.

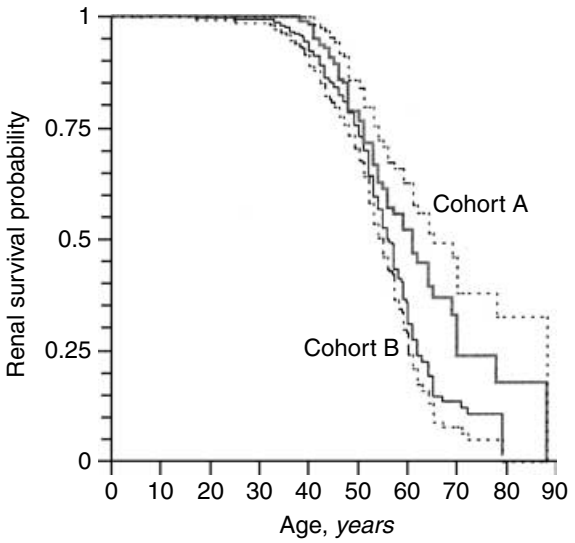


Fig. 1. Survival time to end-stage renal disease (ESRD). The study included 315 patients with autosomal-dominant polycystic kidney disease (ADPKD) from 83 *PKD1* families enrolled in natural history studies at the University of Colorado between 1985 and 2003 (Cohort A, thick line) and 389 relatives from the same families not evaluated clinically (Cohort B, thin line). The survival distributions were significantly different ($P < 0.005$). Upper confidence limits of cohort A and lower confidence limits of cohort B are shown as dotted lines.

and family i. Maximum likelihood estimators of the mean squares corresponding to different variance components were obtained using the GLM procedure in SAS [35]. Significance levels were determined assuming F distributions for the F ratios comparing between- and within-family mean squares (family effect) in model 1, and for the F ratios comparing between-family and between-sibship mean squares (mutation effect) and comparing between- and within-sibship mean squares (modifier gene effect) in model 2.

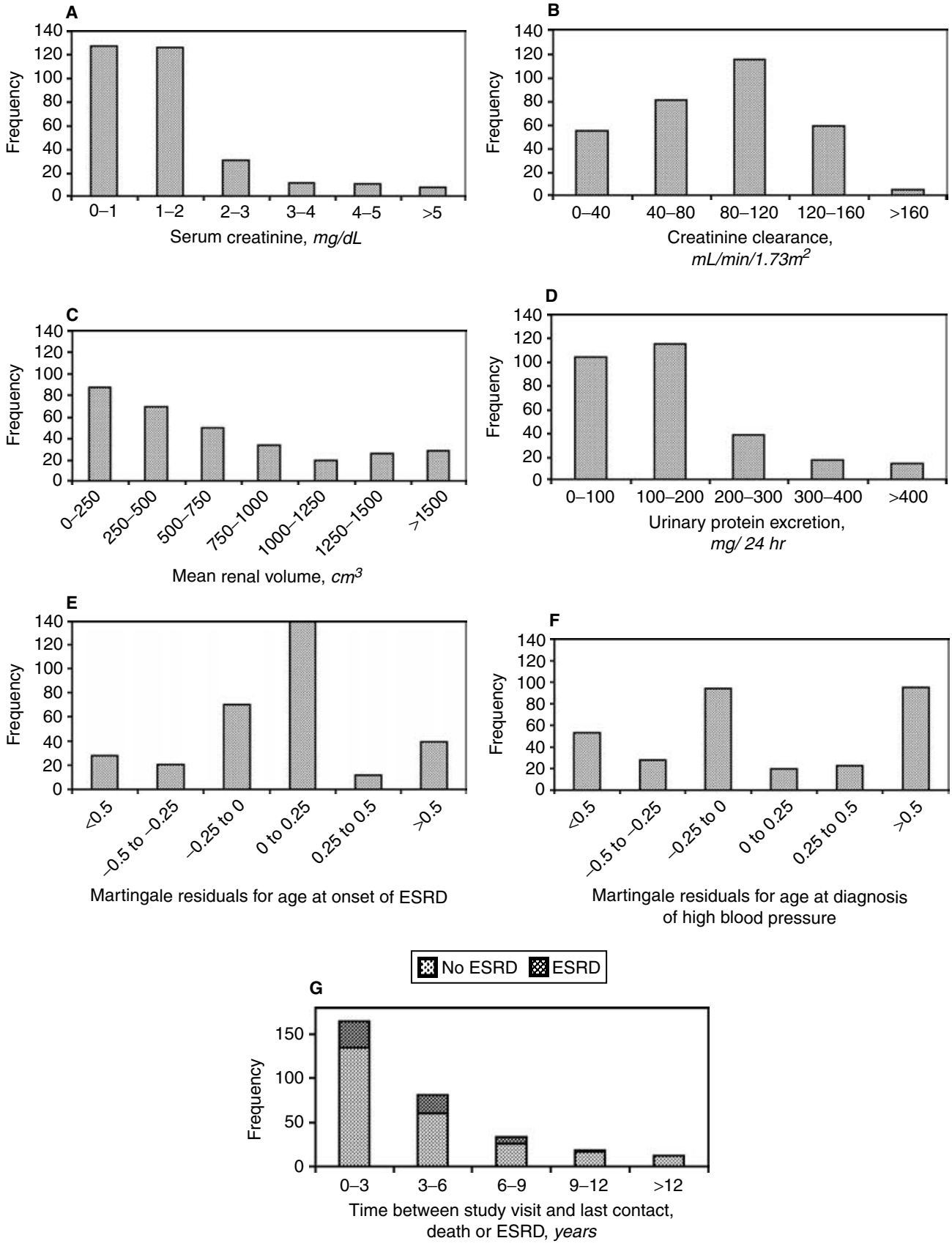
Heritability (broad sense) is defined as the ratio of the genetic component of variation to total phenotypic variation. Heritability analyses in the present context provide estimates of the contribution to phenotypic variability of modifying effects in the genetic background. Heritability estimates were calculated using SOLAR, [40] with age and gender included as covariates. All PKD1-affected relatives within a family were connected through their common ancestors in this analysis; however, all phenotypes for affected relatives who did not participate in natural history studies, and phenotypes for unaffected spouses, were coded as unknown.

RESULTS

Characteristics of the family resource

A detailed description of the patient and family resource and documentation of their classification as PKD1 are shown in Table 1. Family sizes ranged from one to 32 total affected relatives, of which one to 17 affected relatives per family were examined in natural history studies. There were one to 16 sibships per family with at least one affected relative, and one to 12 sibships in which at least one affected relative was examined.

Characteristics of the study population are shown in Table 2 and in Figures 1 and 2. None of the 315 affected relatives (cohort A) enrolled in natural history studies had renal failure at the time of their examination; however, 61 (19%) progressed to ESRD within 1 to 10 years following their examination, at a median age of 61 years. The 389 additional relatives (cohort B) were older than the clinically evaluated subjects (mean year of birth 1940 versus 1961), with 180 (46%) having reached ESRD at a median age of 56 years. Kaplan-Meier renal survival curves for the two populations are shown in Figure 1. The 5-year difference in median survival between younger and older cohorts from the same families is consistent with previous studies demonstrating improvements in renal survival in recent years [10]. Means and standard deviations for the other phenotypes in the clinically evaluated subjects are shown in Table 2. All of the phenotypes showed significant age effects. Liver cysts were present in nearly half (48%) of the patients. Females with ADPKD tended to have more liver cysts than males, but these differences did not reach statistical significance in this population with a mean age of 34 years. Over half (59%) of the patients were diagnosed with hypertension before


A
Frequency
0–1
1–2
2–3
3–4
4–5
>5
Serum creatinine, mg/dL
B
Frequency
0–40
40–80
80–120
120–160
>160
Creatinine clearance, mL/min/1.73m2
C
Frequency
0–250
250–500
500–750
750–1000
1000–1250
1250–1500
>1500
Mean renal volume, cm3
D
Frequency
0–100
100–200
200–300
300–400
>400
Urinary protein excretion, mg/ 24 hr
E
Frequency
<0.5
−0.5 to −0.25
−0.25 to 0
0 to 0.25
0.25 to 0.5
>0.5
Martingale residuals for age at onset of ESRD
F
Frequency
<0.5
−0.5 to −0.25
−0.25 to 0
0 to 0.25
0.25 to 0.5
>0.5
Martingale residuals for age at diagnosis of high blood pressure
No ESRD
ESRD
G
Frequency
0–3
3–6
6–9
9–12
>12
Time between study visit and last contact, death or ESRD, years

(46%), after (3%), or at the time of the clinic visit (10%). The mean age at diagnosis of hypertension was 33 years, with males on average diagnosed at younger ages (mean 31 years) than females (mean 35 years). There were also significant gender differences in serum creatinine concentration and renal volume; however, there were no significant gender differences in age at onset of ESRD, urinary protein excretion, or creatinine clearance.

Frequency distributions for the 315 relatives enrolled in natural history studies are shown in Figure 2, including duration of follow-up and martingale residuals for age at diagnosis of hypertension and age at onset of ESRD. Serum creatinine, creatinine clearance, renal volume, urinary protein excretion, and liver cyst phenotypes were measured at the time of the clinic visit. Age at diagnosis of hypertension was obtained at the time of the clinic visit and upon follow-up. As mentioned, none of these relatives were in ESRD at the time of the clinic visit. For patients who developed ESRD later, the time since the clinic visit is highlighted on the frequency distribution of duration of follow-up. Most of the frequency distributions showed significant skewness and leptokurtosis. Normalizing transformations reduced skewness, but had a limited effect on kurtosis. In theory, the hypothesis tests depend on normality; however, in practice, the dependence decreases with increasing sample size. In order to assess the effects of nonnormality with the current sample size, the same analyses were applied to data with and without transformation. The results were remarkably similar, indicating the analyses are robust against deviations from normality given the large number of patients included in the study.

Model 1 and model 2 ANOVA

Results for model 1 and model 2 ANOVAs on normalized data are shown in Table 3. When appropriate (Table 2), age and gender were included as covariates in the analyses. Model 1 analyses replicate other studies in which between-family variation was compared to within-family variation without regard to the relationships between different family members [14–17]. Consistent with the results of those studies, there is significant evidence for increased variation between families compared to variation within families for age at diagnosis of hypertension and age of onset of ESRD. We additionally found evidence for significant between-family phenotype differences with respect to creatinine clearance, urinary protein excretion, renal volume, and the presence/absence and number of liver cysts, but not for serum creatinine.

Table 3. Results of model 1 and model 2 analyses of variance of clinical phenotypes for 315 affected relatives from 83 *PKD1* families (age- and gender-corrected)

	Model 1 Effect (P value)	Model 2 Effect (P value)	
Phenotype	Family	Mutation	Modifier genes
Serum creatinine *mg/dL*	0.1502	0.7410	0.0045
Creatinine clearance *mL/min/1.73 m²*	0.0185	0.3823	0.0126
Urinary protein excretion *mg/24 hours*	0.0069	0.2278	0.0059
Renal volume cm^3	0.0212	0.2845	0.0042
Presence/absence of liver cysts	0.0171	0.1999	0.0286
Number of liver cysts	0.0034	0.2103	0.0008
Age at diagnosis of hypertension (martingale residuals)	0.0008	0.0787	0.0045
Age of onset of ESRD (martingale residuals)	0.0026	0.1512	0.0029

ESRD is end-stage renal disease.

Model 2 analyses focus on the pattern of intrafamilial phenotype differences to determine the contribution to between-family effects of differences in the nature of the *PKD1* mutation (mutation effects) and differences in genetic background (modifier effects). Since all members of the same family share the same *PKD1* mutation, the characteristics of different *PKD1* mutations may contribute to phenotypic differences between families, but does not contribute to phenotypic differences within families. However, since members of the same sibship share their genetic background to a greater extent than members of different sibships within the same extended family, modifier alleles contribute to intrafamilial variability in addition to interfamilial variability.

As shown in Table 3, there was no evidence for differential effects of different mutations on any of the phenotypes studied (i.e., the between-family mean squares were not significantly higher than the between-sibship mean squares). On the other hand, inherited modifier effects were significant ($P < 0.05$), and in some cases,

Fig. 2. Frequency distributions of clinical characteristics of 315 subjects with autosomal-dominant polycystic kidney disease (ADPKD) from 83 *PKD1* families. Graphs (*A*) through (*F*) show data from the subjects' natural history visits, at which time no subject had reached end-stage renal failure (ESRD). (A) Serum creatinine concentration 1.5 ± 1.2, median 1.1, range 0.5 to 8.1 mg/dL. (B) Creatinine clearance 83 ± 39, median 85, range 9 to 186 mL/min/1.73 m^2. (C) Renal volume (mean of both kidneys) 671 ± 537, median 509, range 71 to 2651 cm^3. (D) Urinary protein excretion 252 ± 393, median 138, range 17 to 3472 mg/24 hours. (E) Martingale residuals for the age at onset of ESRD (or no ESRD) 0 ± 0.4, median 0, range –1.9 to 1.0. (F) Martingale residuals for the age at diagnosis of hypertension (or no hypertension) 0 ± 0.7, median 0, range –4.1 to 1.0. (G) Time between each subject's natural history visit and either reaching ESRD, death without ESRD, or last follow-up without ESRD. Subjects with ESRD 3.3 ± 2.3, median 3.0, range 0 to 10 years; subjects without ESRD 3.6 ± 4.2, median 2.7, range 0 to 17 years.

Table 4. Heritability of clinical phenotypes for 315 affected relatives from 83 *PKD1* families (age- and gender-corrected)

Trait	Heritability	Standard error	*P* value
Serum creatinine *mg/dL*	0.183	0.117	0.0476133
Creatinine clearance *mL/min/1.73 m²*	0.323	0.111	0.0007320
Urinary protein excretion *mg/24 hours*	0.481	0.123	0.0000156
Renal volume *cm³*	0.427	0.112	0.0000090
Presence/absence of liver cysts	0.569	0.187	0.0007362
Number of liver cysts	0.353	0.111	0.0003161
Age at diagnosis of hypertension (truncated sample)[a]	0.462	0.179	0.0045690
Age at diagnosis of hypertension (martingale residuals)[b]	0.585	0.139	0.0000031
Age of onset of ESRD (truncated sample)[a]	0.499	0.297	0.0503910
Age of onset of ESRD (martingale residuals)[b]	0.434	0.153	0.0013154

ESRD is end-stage renal disease.

[a]Analyses restricted to 185 patients with hypertension or 61 patients with ESRD.

[b]Analyses applied to all of 315 affected relatives with clinical data with event-free relatives assigned a score based on their age.

highly significant ($P < 0.01$) (i.e., the between-sibship mean squares were significantly higher than the within-sibship mean squares). Therefore, the pattern and extent of intrafamilial variation is more likely explained by differences in genetic background than by differences in the PKD1 mutation. However, when the additional 389 family members without clinical evaluation were added to the analysis of age of onset of ESRD, mutation effects were highly significant ($P < 0.0005$), but there was also evidence for modifier gene effects ($P < 0.04$).

Heritability

Results of heritability analyses on normalized data are shown in Table 4. An implicit assumption of these analyses is that variation in genetic background is the primary factor in determining phenotype differences between families. Results of model 2 analyses (Table 3) support this assumption. The main purpose of heritability analyses in this context is to estimate the proportion of the total phenotypic variability in PKD1 that can be attributed to variation in inherited modifiers. The estimates of heritability were obtained by fitting a model in which the phenotypic covariance between pairs of relatives is expressed as a function of their kinship coefficient, or the expected proportion of alleles they share on average across the entire genome [40]. For example, the kinship coefficient between pairs of siblings is 1/2 and the kinship coefficient between pairs of cousins is 1/8. Using quantitative genetics theory, it can be shown that the phenotypic covariance between siblings is equal to 1/2 of the genetic variance, and the phenotypic covariance between cousins is equal to 1/8 of the genetic variance.

The pattern of the heritability estimates (Table 4) for different phenotypes corresponds closely to the significance levels in model 1 analyses in Table 3, with serum creatinine showing less evidence for genetic background effects compared to other phenotypes. The similarity in the results of the two analyses is expected since both analyses are tests of between-family phenotype differences. However, by incorporating the precise genetic relationships between members of the same family, the precision and statistical power of the heritability analyses is increased in comparison to the model 1 analyses. All of the phenotypes showed significant ($P < 0.05$) heritability, confirming that one or more modifier genes in the genetic background accounts for a significant fraction of the phenotypic variability seen in patients with a mutation in *PKD1*. The estimated contribution of the genetic background to the observed variation, as measured by the heritability estimates, was as follows: serum creatinine (18%), creatinine clearance (32%), number of liver cysts (35%), renal volume (43%), and urinary protein excretion (48%). The highest heritabilities were seen for the presence/absence of liver cysts (57%), age at diagnosis of hypertension (46% and 59%), and age at onset of ESRD (50% and 43%). Heritability estimates obtained from analyses with all 315 PKD1-affected relatives scored using martingale residuals for age at diagnosis of hypertension and age at onset of ESRD did not differ significantly from the estimates obtained from analyses in which relatives who had not developed hypertension or ESRD were excluded. However, the increased power of the analyses of the complete sample (martingale residuals) is apparent from the lower standard errors and improved significance levels compared to the results for the truncated sample.

DISCUSSION

The interfamilial and intrafamilial phenotypic variability in PKD1 is poorly understood. Many studies have reported extensive interfamilial phenotypic variability for hypertension and progression to ESRD [11–14]. In addition to confirming these results, we found significant between-family differences in preclinical markers and liver cysts in 315 PKD1 patients without ESRD (Table 3, model 1). Either or both of two hypotheses could explain these results. One hypothesis is that the phenotype depends on the nature of the *PKD1* mutation, which typically differs in different families (Table 3, model 2, mutation effects). This would explain phenotypic differences between families, but would not explain phenotypic differences within families, since all members of the same family share the same mutation. Another hypothesis is that inherited modifiers in the genetic background, which differ both between and within families, modulate the expression of the *PKD1* mutation (Table 3, model 2, modifier gene effects). This would explain phenotypic

differences within families in addition to phenotypic differences between families. The two hypotheses are not mutually exclusive (i.e., the renal and extrarenal manifestations and severity of PKD1 could depend on both the mutation and the genetic background).

We found significant evidence that inherited modifiers in the genetic background are major factors in the intrafamilial and interfamilial phenotypic variability in preclinical manifestations, complications, and severity of PKD1. Specifically, our results demonstrate that phenotypic variability between patients with the same mutation and different genetic backgrounds (remote relatives; between-sibships mean square) is significantly increased in comparison to patients with the same mutation and a more similar genetic background (siblings; within-sibships mean square). Our results also show that phenotypic variability among patients with different mutations and different genetic backgrounds (unrelated patients; between-family mean square) was not significantly different in comparison to patients with the same mutation and different genetic backgrounds (remote relatives; between-sibships mean square) for most phenotypes.

The majority of *PKD1* mutations encode a truncated polycystin-1 protein [18, 19, 41–43], the functional consequences of which are largely unknown, although there is indirect evidence that the manifestations and/or severity of PKD1 may depend on the length of the truncated protein [18–20]. In one study [19], progression to ESRD was more rapid in patients with mutations at the 5′ end of the gene compared to patients with mutations at the 3′ end of the gene, suggesting a truncated polycystin-1 protein may retain some residual function, depending on its length. However, consistent with our results (Table 3, model 2) the magnitude of the difference was small (median time to ESRD 53 and 56 years for 5′ and 3′ mutations) in relation to the total variation in age at onset of ESRD (<5% assuming a standard deviation of 10 years), and in relation to the 5-year difference in median renal survival between younger and older cohorts from the same families. Since the study population and source of the data differed from ours, we replicated their study design by applying our analyses (model 2) to retrospective and prospective family history data for renal survival from 704 total living and deceased relatives from 83 PKD1 families (Table 1). Mutation effects were highly significant ($P < 0.0005$) in the total group of 704 relatives, but there was also evidence for modifier gene effects ($P < 0.04$). Upon inspection of the family data, we attributed these results to cohort differences in renal survival combined with a clustering of older-aged and deceased relatives within a few large families. However, this would not explain other evidence for positional effects of *PKD1* mutations [19, 20]; and we cannot rule out the possibility that our reported analyses are underpowered for detecting mutation effects, due to the smaller sample size, the young age of the study population, and relatively short duration of prospective follow-up (Fig. 2).

The results of heritability analyses indicate the genetic background contributes 18% to 59% of the total (genetic and non-genetic) phenotypic variation in renal volume and function, proteinuria, liver cyst phenotypes, age at diagnosis of hypertension, and subsequent progression to ESRD in the study population. It should be emphasized, however, that these percentages are estimates which must be viewed with circumspection, as indicated from the high standard errors (Table 4). In addition, heritability estimates do not allow for similarity between relatives due to similarities in environment. However, the estimates appear reasonable in indicating that the genetic background accounts for a lower fraction of the variability in serum creatinine (18%) and creatinine clearance (32%) than is seen for other phenotypes, indicating a stronger influence of nongenetic factors on these measures in individuals with relatively normal renal function. Renal volume displays remarkably high heritability (43%), despite the well-known limitations of ultrasound for determining the size and number of renal cysts [44]. The intraclass correlation coefficient for multiple ultrasound readings at our institution was 0.86, decreasing to 0.34 for kidneys greater than 1000 cm^3. Nonetheless, renal volume, even as measured by ultrasound, correlates consistently with other measures of disease progression [6, 7].

The concept of heritability is widely used in genetics; however, there is often confusion about the biologic significance of heritability estimates. Heritability analyses are typically applied to normal variation in quantitative phenotypes or to susceptibility to common diseases. As shown in the present study, the same principles can be applied to estimate the contribution of the genetic background to phenotypic variability in the manifestations and severity of *PKD1* mutations; however, the interpretation and the strengths and limitations of heritability analyses remain the same. Most important, heritability estimates are ratios that often differ between different populations, between correlated phenotypes, and for the same phenotype measured at different points in time, whether or not the underlying genetic pathways and mechanisms are the same. These differences often, but not always, reflect differences or changes in nongenetic factors, which are represented only in the denominator of the ratio. For example, the somewhat lower heritability of serum creatinine compared to the heritability of creatinine clearance, although not statistically significant, most likely reflects intra- and interindividual variation in nongenetic factors such as diet and hydration, which have a larger effect on serum creatinine than on creatinine clearance.

The main utility of the heritability estimates (Table 4) from the present study is in providing important baseline information about the genetic architecture behind the

phenotypic expression of PKD1. Heritability estimates obtained from this study are most useful for assessing the efficiency of family-based linkage and association studies to map and identify specific modifier genes that contribute to phenotypic differences in various markers of disease progression observed prior to the onset of ESRD. To date, association studies of candidate modifier genes, such as the *ACE* gene, which encodes angiotensin-converting enzyme (ACE), and the *ENOS* genes, which encode endothelial nitric oxide synthase (eNOS), have met with limited success [45–56]. The conflicting results of these studies could reflect genetic or other differences in the populations studied, or the relatively small effects of these genes in relation to other modifying effects. However, a more likely explanation is that candidate gene studies are constrained, on the one hand, by current gaps in understanding the molecular pathology of PKD1 and the vast number of potential candidates for inherited modifiers, and, on the other hand, by current gaps in understanding the functional significance of allelic variation in known genes, and by the overwhelming number of genes that remain uncharacterized in terms of either allelic variation, structure, or function. Quantitative trait locus (QTL) linkage mapping is an appealing alternative to candidate gene association studies in that it does not require any knowledge of disease pathogenesis or gene function. Although significant heritability is a prerequisite for QTL mapping, the power of the approach is also dependent on the number of different modifier loci that contribute to phenotypic variability, the magnitude and nature of effects at any given locus, and other factors that cannot be inferred from heritability estimates. Other considerations may be unique to a particular application, including the nature of the phenotype and the size and structure of the families available for study.

There are clear indications favoring QTL mapping in large PKD1 pedigrees, based primarily on the need to distinguish PKD1 and PKD2 families prior to analyses. Beyond this, the power and efficiency of the QTL approach depends on assumptions about how many different modifier loci contribute to the heritable variation in the phenotypic expression of PKD1, and how large is the effect at any given locus. Observations of extreme differences in the severity of PKD1 among members of the same family have led other investigators to hypothesize that phenotypic variation could be explained by one or a few modifier loci [29]. Our results showing significant phenotype differences between distant relatives, in relation to close relatives (Table 3, model 2, modifier effects) but not in relation to unrelated patients (Table 3, model 2, mutation effects), provide additional support for this hypothesis.

We used computer simulation to determine the expectations for QTL mapping in 234 relatives from the seven largest families from our resource (>15 total affected relatives, families 109, 110, 165, 579, 581, 1313, 1359) (Table 1), assuming a modifier locus contributes 50% of the phenotypic variability and assuming a modifier locus contributes 15% of the phenotypic variability. Based on 500 simulated replicates of these families, it will require 10 to 20 total families and genotype data from 400 to 700 total affected and unaffected relatives to map a modifier locus that contributes 50% of the phenotypic variability. The wide range for the sample size estimates reflects different assumptions about allele frequencies and genotypic effects and considerable variability in linkage outcomes between different replicates of the same family. The sample size expectations increase exponentially as the contribution to phenotypic variability decreases; for example, it will require 100 to 200 total families with 4000 to 8000 total relatives to map a modifier locus that contributes 15% of the phenotypic variability.

CONCLUSION

The present study has demonstrated that interfamilial and intrafamilial phenotype differences in PKD1 are substantially influenced by differences in genetic background, and that inherited modifiers account for an estimated 18% to 59% of the phenotypic variability in PKD1. Although the nature and size of phenotypic effects of individual modifier genes remain unclear, the extreme intrafamilial variability in the phenotypic expression of PKD1 is consistent with the hypothesis that one or a few modifier loci have a major effect on the progression and severity of PKD1. These results, in turn, support other evidence that a limited number of major genes often account for a surprisingly large fraction of the variability in quantitative phenotypes [57–59]. We conclude that the effects of individual modifier genes may be large enough to be of clinical significance, and, as such, important enough to proceed with mapping and identifying specific modifier genes by QTL linkage analysis and positional cloning.

The focus on markers of disease progression prior to the onset of ESRD is especially important in clarifying the extent and sources of variability in the subgroup of subjects most likely to participate in prospective studies.

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